

- 6 Hornung, H., Raviv, D., and Krumgalz, B.S., Mar. Poll. Bull. 12 (1981) 389.
- 7 Russel, G., and Morris, O.P., Nature 228 (1970) 288.
- 8 Bryan, G.W., and Hummerstone, L.G., J. mar. biol. Ass. U.K. 53 (1973) 839.
- 9 Bradshaw, A.D., in: Effects of air pollutants on plants. Ed. T.A. Manfield. Soc. exp. Biol. Seminar 1. Cambridge University Press (1976).
- 10 Lavie, B., and Nevo, E., Mar. Ecol. (submitted, 1984).
- 11 Kettlewell, H.B.D., The evolution of melanism. Clarendon Press, Oxford 1973.

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Antihepatotoxic constituents of *Garcinia kola* seeds

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Summary. Kolaviron, a fraction of defatted methanolic extract and biflavanones of *Garcinia kola* seeds significantly antagonized the lethal poisoning of mice with phalloidin. *Garcinia* biflavanones GB1, GB2 and kolaflavanone were isolated as the active constituents.

Key words. Kolaviron; antiphalloidin; biflavonoids; *Garcinia kola*; antihepatotoxic.

Extracts and whole seeds of *Garcinia kola* Heckel gave remarkable improvement of liver function in patients with chronic hepatitis and cholangitis after treatment for 14 days at a Nigerian herbal home¹. Since there are no pharmacological studies to support the use of this plant in African ethnomedicine, the extract and isolates of *G. kola* have been subjected to various tests to determine any possible protective role on the liver. Previous phytochemical investigation of *G. kola* resulted in the isolation and characterization of cycloartenol, and 24-methylene cycloartenol from the light petroleum extract²; C-3/8"-link biflavanones GB1, GB2, GB1a and kolaflavanone from the ethylacetate extract of the seeds³. These biflavanones and their glycosides have also been isolated from the stem bark⁴. The ether soluble fraction of the alcoholic extract yielded apigenin-5,7,4'-trimethyl ether, apigenin-4'-methylether, fisetin, amentoflavone, kolaflavanone and GB1⁵. Waterman and his colleagues have also reported the antimicrobial properties of a benzophenone, kolanone, isolated from the light petroleum extract of *G. kola* seeds⁶.

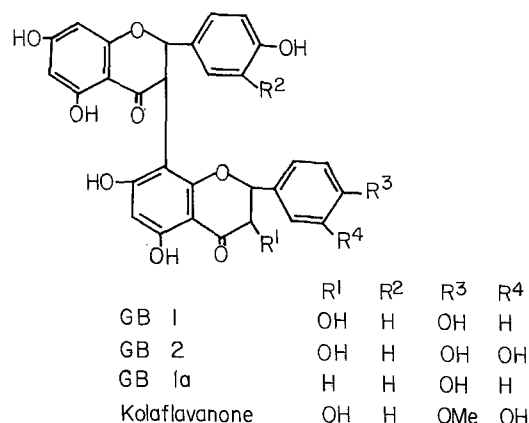
This communication describes the antihepatotoxic properties of kolaviron (a fraction of the defatted alcoholic extract) and isolates of *G. kola* seeds as evidenced by liver protection of laboratory animals challenged with phalloidin, a known liver toxin.

Materials and methods. Seeds of *Garcinia kola* Heckel (Fam. Guttiferae) were collected from a cultivated tree at Umukabia (Mbano, Nigeria) in November 1980. A voucher specimen has been deposited at the Pharmacy Herbarium at the University of Nigeria, Nsukka.

20 kg of powdered seeds of *G. kola* were extracted with light petroleum spirit (b.p. 40–60°C) and then methanol in a soxhlet. The petrol extract, which showed no antihepatotoxic activity, was discarded. The methanolic extract was concentrated under reduced pressure and extracted with petrol. The defatted alcoholic extract was partitioned between chloroform and water. The chloroform extract on evaporation of the solvent gave a golden-yellow powder, kolaviron. Thin layer chromatography of this substance revealed the presence of three main compounds. Kolaviron was separated by Droplet Counter Current Chromatography using chloroform-methanol-water (7:13:8) as the solvent system (the more polar upper layer as the mobile phase). The mobile phase was delivered at a regulated pressure of 8 psi and the eluates collected in 15 ml fractions. Biflavanones GB1 (II-3-I-4'-II-4'-I-5-II-5-I-7-II-7-hepta-hydroxy-3/8"-biflavanone), GB2(II-3-II-3'-1-4'-II-4'-1-5-II-5-1-7-II-7-octahydroxy-3/8"-biflavanone) and kolaflavanone (I-3'-II-3-I-4'-II-4'-I-5-II-5-I-7-II-7-octahydroxy-II-3'-methoxy-3/8"-

biflavanone) were identified from the analysis of their spectral data (UV, IR, MS, H and ¹³C-NMR) and direct chromatographic comparison with reference compounds. The biflavanone mixture, kolaviron and the isolates were suspended in normal saline with Tween 20.

Biological methods. 240 female Swiss mice with an average weight of 24 g were maintained on laboratory chow and tap water ad libitum. Test substances in 10 ml/kg at stated doses (table) were given by intraperitoneal injection and followed 1 h later with 3 mg/kg of phalloidin (Sigma Chemical Company,



Antiphalloidin action of extract and isolates of *G. kola* in female mice

Substance	Dose mg/kg i.p	Number of animals	Survival ¹ rate (%)
Control (phalloidin) only	3.0	20	5
Vehicle control	3.0 phalloidin and 10 ml Tween 20 in normal saline	20	10
Kolaviron	10	20	30
Kolaviron	50	20	50
Kolaviron	100	20	100
GB1	10	20	50
GB1	50	20	85
GB1	100	20	100
GB2	10	20	50
GB2	50	20	100
Kolaflavanone	10	20	35
Kolaflavanone	50	20	100

¹p = 0.05 in all treated groups against toxin and vehicle control group.

Poole) administered intraperitoneally. Control groups received phalloidin and/or Tween 20 in normal saline. The death rate was determined on the seventh day.

Five animals per group were sacrificed by cervical dislocation and blood samples were collected to study the serum activities of glutamic pyruvic transaminase (GTP), glutamic oxaloacetic transaminase (GOT), acid phosphatase, and B-glucuronidase by employing the methods described by Tuchweber⁷. Liver tissues from five mice from each group were treated and examined⁷ for gross morphological characters. The statistical significance of the differences between various dose regimens was evaluated by analysis of variance, and the χ^2 -test was used to evaluate the treated groups against the controls.

Phalloidin, a toxic constituent of the death-cup toadstool *Amanita phalloides* causes fatal hepatic injury in experimental animals. In small rodents, death of the animals occurs within 5 h; the animals in this study were observed for an extended period of seven days to establish absence of delayed phalloidin toxicity. It has been established that the hepatocytic plasma membrane is the primary site of action of phalloidin⁸; the lethal effect is a sequel to the observed interaction of the toxin with membrane-associated microfilaments.

As shown in the table, kolaviron and the isolated biflavanones completely protected the mice from the lethal effect of phalloidin. The antagonistic activity of kolaviron could be due to an inhibition of phalloidin binding to the cell surface. Although it is well known that certain compounds capable of protecting mice against lethal doses of phalloidin are not necessarily hepatoprotective compounds, the antihepatotoxic activity of kolaviron is substantiated by the results of the biochemical and histological studies. Pretreatment with a single dose of kolaviron completely abolished the morphological changes induced by the toxin, as evidenced by light microscopic examination of liver tissue from poisoned mice, and the activities of the serum enzymes (GPT, GOT, acid phosphatase and B-glucuronidase) were significantly decreased. Kolaviron when given alone did not result in changes of hepatocytic structure or serum enzyme activities. Details of the activity of serum enzymes, and

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morphological characteristics, have been reported elsewhere⁹. It is unlikely that the antiphalloidin activity of kolaviron is due to binding of the biflavanones to phalloidin in the peritoneum and inhibition of the delivery of the toxin to the liver, because spectroscopic studies showed no interaction between the two molecules.

This work provides evidence to support the use of kolaviron and Garcinia biflavanones as potent antihepatotoxic agents. Further work is required to establish the protective ability of these biflavanones on animals poisoned with other hepatotoxins, and the potential benefits of kolaviron or its constituents in the treatment of hepatitis.

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- 1 Iwu, M.M., unpublished evaluation; Patent Application 426/82, Federal Republic of Nigeria (1982).
- 2 Aplin, R. T., Blasdale, J. K. C., Halsall, T. G., and Hornby, G. M., J. chem. Soc. C (1967) 246.
- 3 Cotterhill, P. J., Scheinmann, F., and Stenhouse, I. A., J. chem. Soc. Perkin Trans. I (1978) 532.
- 4 Iwu, M. M., Planta Medica 45 (1982) 147.
- 5 Iwu, M. M., and Igboke, O. A., J. natn. Prod. (Lloydia) 45 (1982) 650.
- 6 Hussain, R. A., Parimoo, A. G., and Waterman, P. G., Planta Medica 44 (1982) 78.
- 7 Tuchweber, B., Sieck, R., and Trost, W., Tox. appl. Pharmac. 51 (1979) 265.
- 8 Frimmer, M. Phalloidin, a membrane specific toxin, in: Pathogenesis and Mechanism of Liver Cell Necrosis, p.163. Ed. D. Keppler. MTP Press, Lancaster 1975.
- 9 Iwu, M. M. Antihepatotoxicity of Biflavonoid of *Garcinia kola* Heckel. Proceedings of 32nd Annual Congress for Medicinal Plant Research, Antwerp 1984; Farmaceutisch Tijdschrift Voor België 61 (1984), 248.

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